

The results of this test should be interpreted and utilized after review of the following specifications:

Product Indication

The PGxPredict:RITUXIMAB test is intended to identify a patient's *FCGR3A* genotype from genomic DNA extracted from a whole blood sample. This information can be used to aid clinicians in developing a treatment plan for their patients with follicular non-Hodgkin's lymphoma.

Description of Genetic Assays

Gene: *FCGR3A*
 Nucleotide Change: 4985G>T
 Amino Acid Change: Phe158Val
 Reference Sequence ID: rs396991

Background

The PGxPredict:RITUXIMAB test detects a single nucleotide polymorphism (rs396991) in the *FCGR3A* gene that has been correlated with overall response rate and progression-free survival in patients with treatment of follicular non-Hodgkin's Lymphoma (NHL) treated with rituximab monotherapy.^{1,2} The *FCGR3A* gene encodes a receptor found on the surface of immune effector cells that binds therapeutic antibodies of the IgG1 subtype. Effector functions, including antibody-dependent cellular cytotoxicity (ADCC), are triggered when antibodies bind *FCGR3A* receptors, resulting in cross-linking of effector cells to target cells, such as CD20-positive B cells in NHL. Both rituximab binding affinity and target cell lysis are influenced by the genotype of *FCGR3A*.^{3,4} These genotype correlations are expected to extend to other monoclonal antibodies of the IgG1 subtype that may depend on ADCC for their therapeutic efficacy.

Description of Methods

- Sample Acquisition:** Whole blood is collected via finger stick on FTA® Cards (Whatman®, Middlesex, UK) from which genomic DNA is isolated. Bar code identifiers are used to identify and track samples through a laboratory information management system.
- DNA Sequence Analysis:** The DNA is amplified by polymerase chain reaction (PCR), and is used to generate a template for direct sequencing.

The amplicon is produced in at least two independent PCR reactions and direct sequencing is performed in both forward and reverse directions using dye-terminator chemistries. Automated electrophoretic separation of sequencing reactions is performed. At least two reverse reads are required for the region for variant analysis.

- Variant Detection:** Sequence traces are analyzed for heterozygous or homozygous variants with respect to a publicly available reference sequence. The patient and reference traces are generated using the same protocol. Patient sequence traces are compared with reference traces to validate variant results. Two trained technicians independently score all traces for variants, and a trained supervisor reconciles all discrepancies.
- Report Generation:** The *FCGR3A* genotype is reported, and patients are classified as Average (158V/F, 158F/F) or High (158V/V) likelihood of response according to the table below. The final report is reviewed and signed by a CLIA-licensed Laboratory Director.

Performance Characteristics

Analytical Accuracy: The test is 99.8% accurate in identifying the genotype of the 4985G>T polymorphism in *FCGR3A*. The chance of an inaccurate genotype is minimized by requiring two independent sample preparations that are processed in parallel and two trained technicians to independently examine each trace. Chances of inaccurate results are further minimized by the use of a validated sample tracking system that uses robotics and bar code identifiers.

This test was developed and its performance characteristics determined by PGxHealth, LLC. FDA approval is not currently required for clinical use of this test. Validation was done as required by the Clinical Laboratory Improvement Amendments of 1988 (CLIA) (CLIA License Number: 07D0995237; State License Number: CL-0633).

Test Interpretation

Based on data from 136 patients in two independent cohorts^{1,2}, the *FCGR3A* 4985G>T genotype is utilized to classify patients as Average or High Likelihood of Response to rituximab monotherapy in the treatment of follicular non-Hodgkin's lymphoma.

The Genotypes Will be Interpreted According to the Following Table:

Nucleotide Change	Amino Acid Change	Effect on Response to Rituximab Monotherapy
4985 G/G	158 V/V	High Probability of Response
4985 G/T	158 V/F	Average Probability of Response
4985 T/T	158 F/F	Average Probability of Response

References

- G. Cartron et al., *Blood* 99, 754 (2002).
- W. K. Weng, R. Levy, *J. Clin. Oncol.* 21, 3940 (2003).
- S. Dall'Ozzo et al., *Cancer Res.* 64, 4664 (2004).
- H. R. Koene et al., *Blood* 90, 1109 (1997).



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